independent of lignocellulosic waste source. In their process the irradiated samples were treated with Meicelase (Meiji Seika Industry, Co., Ltd.) at a 2% substrate concentration, 0.2% enzyme concentration, followed by incubation for 48 h. Differences existed between enzymatic treatments in the two processes and may account for the differences observed (Faithful, 1984).

In the study reported here, temperature was not measured; however, the typical dark brownish color was apparent, indicating caramelization of the sugars released during microwave treatment. Samples had a characteristic burned smell. The preliminary results presented indicate that microwave treatment reduced the in vitro digestibility of the agricultural wastes evaluated. The lack of an observed enhancement in IVDMD may be due to relatively low microwave power settings since there is a poor coupling of microwave energy to lignin (Chan and Krieger, 1981) although less treatment time is required for lignocellulosic wastes than for woody plants (Azuma et al., 1984).

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Nitrite-Tryptophan Reaction: Evidence for an Equilibrium between Tryptophan and Its Nitrosated Form

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Nitrite reacts with *N*-acetyltryptophan (NacTRP) in aqueous solution to yield *N*-acetyl-N^1-nitrosotryptophan (NacNOTRP), which was found to be unstable. The reaction was followed by means of UV spectrometry using different combinations of the operating parameters (relative concentrations, pH, temperature). It was concluded from the collected data that an equilibrated reaction takes place between NacTRP, nitrite, and NacNOTRP. The reacting species of nitrite was found to be HNO_2. The value of the equilibrium constant equals 455.6 ± 0.13 M^{-1}. The temperature effect study yielded $\Delta H_0 = -54\,000$ J·mol^{-1} and $\Delta S_0 = -140$ J·mol^{-1}·K^{-1}. These results provide an insight on the release of nitrite observed on cured meat and myofibrillar proteins and the "transnitrosation" reactions that occur from protein tryptophan residues toward myoglobin heme groups.

Nitrite in cured meat products is responsible for color stabilization and bacteriostatic protection and induces a characteristic flavor. The color mechanism is known as the fixation of one or two NO on the heme group of the myoglobin (Tarladgis, 1962), and some explanations have been given for the bacteriostatic effect (Yarbrough et al., 1980; Reddy et al., 1983). A better knowledge of the reaction mechanisms of nitrite with meat constituents is required for a more precise understanding of the role of nitrite in the development of the characteristic flavor of treated meat products and for preventing the formation of nitrosamine in cured meat. Studies have been made on the distribution of nitrite in the different meat fractions, showing that, during the curing process, the loss of the added nitrite is considerable. Cassens et al. (1977) reported a 5-20% recovery of the initial $Na^{15}NO_2$ in meat systems as free nitrite, but 20-30% of the ^{15}N was bound to protein fraction. Woolford et al. (1976) reported that 30% of the nitrite added to a bovine serum albumin solution could bind to the protein. Noël et al. (1981) observed a release of nitrite from nitrosated meat and myofibrillar proteins after washing with water. The nitrite recovery between first and last washing increased when the incubation pH decreased. The authors concluded an equilibrium exists between a part of bound nitrite and free nitrite. Reactivity of the side chains of protein has been studied for a long

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Table I. Schematic Diagram Showing the Different Couples (Buffer-*N*-Acetyltryptophan Concentration) Used in the pH Dependence Test

	pH 1–2.2	pH 2.5–3.9	pH 3.6–5.2
Buffer	HCl - KCl 0.05 M	ACONITIC ACID - NaOH 0.05 M	ACETIC ACID - SODIUM ACETATE 0.1 M
[NacTRP]	0.12×10^{-3} M	0.12×10^{-3} M	0.24×10^{-3} M

time. A review of work pertinent to the reactivity of amino acids with nitrite has been made by Bonnett and Nicolaidou (1977). Philpot and Small (1938) noted a fast reaction of nitrite with tryptophan but were unable to identify the final products. Bonnett and Holleyhead (1974) described the reaction product of *N*-acetyl-DL-tryptophan methyl ester and nitrite as the *N*-acetyl-DL-N^1-nitrosotryptophan methyl ester. Nakai et al. (1978) and Ito et al. (1983) mentioned this product is not stable. The present work was undertaken in order to provide a better insight on the effect of relative concentrations, pH, and temperature on the reactivity of tryptophan with nitrite.

MATERIALS AND METHODS

Chemicals. The N^α-acetyl derivative of L-tryptophan (NacTRP) was purchased from Sigma (München), sodium nitrite was from Merck, aconitic acid, hydrochloric acid, acetic acid, and potassium chloride were from Carlo Erba, and sodium acetate and sodium hydroxide were from Prolabo (Paris).

Instrumentation. All optical densities were recorded with a double-beam UV spectrometer JOBIN-YVON 100-80 A fitted with thermostating equipment.

Operating Conditions. The different conditions used for the relative concentration, pH, and temperature dependence studies are outlined in Table I. Initial NacTRP concentrations were selected to obtain a measurable amount of N^α-acetyl-N_1-nitrosotryptophan (NacNOTRP). As the concentration of this compound decreased when the pH increased, NacTRP was doubled for the highest pH values. The temperature measured directly in the spectrometer cell was 23 ± 1 °C except during the temperature effect study.

Procedure. *Relative Concentrations and pH Effects Study.* For each pH value three buffered solutions were prepared in a ratio [nitrite]/[NacTRP] of 50/1, 100/1, and 300/1. NacNOTRP formation was followed by measuring the optical density at 335 nm, maximum of the specific absorption band (Kurosky and Hofmann, 1972). Incubation time was 1 h. This corresponds to the maximum of the curve representing the absorption at 335 nm as a function of time. The absorption decreases after that time on account of the instability of the nitrite in solution.

The equilibrium studied was

$$\text{NacTRP} + \text{nitrite} \overset{K_{app}}{\rightleftharpoons} \text{NacNOTRP} \quad (1)$$

"Nitrite" is all the possible species in aqueous solution formed from sodium nitrite (Ridd, 1961). At a given pH, the optical densities OD_1 and OD_2 of two solutions with two ratios [nitrite]/[NacTRP] of 50/1 and 100/1 were recorded. If we assume that an equilibrium exists, it is possible to calculate a molar extinction coefficient (ϵ) for the NacNOTRP from the systems

$$K_{app} = \frac{OD_1/\epsilon}{[\text{nitrite}]_1([\text{NacTRP}]_{in} - OD_1/\epsilon]} \quad (2)$$

ratio 50/1

$$K_{app} = \frac{OD_2/\epsilon}{[\text{nitrite}]_2([\text{NacTRP}]_{in} - OD_2/\epsilon]} \quad (3)$$

ratio 100/1

Then

$$\epsilon = \frac{OD_1 OD_2[[\text{nitrite}]_1 - [\text{nitrite}]_2]}{[\text{NacTRP}]_{in}[OD_2[OD_2[\text{nitrite}]_1 - OD_1[\text{nitrite}]_2]} \quad (4)$$

where K_{app} is the apparent equilibrium constant (depending on the pH), $[\text{NacTRP}]_{in}$ is the initial NacTRP concentration, and $[\text{nitrite}]_{1or2}$ is the initial sodium nitrite concentration in the solution. This concentration was taken as a constant since nitrite was in large excess.

Thus, considering the value of ϵ, the apparent constant K_{app} can be expressed as

$$K_{app} = \frac{OD_{1or2}}{\epsilon[[\text{NacTRP}]_{in} - OD_{1or2}/\epsilon][\text{nitrite}]_{1or2}} \quad (5)$$

The value of this equilibrium constant can be verified by applying it to the following equation for the calculation of the theoretical value of a third optical density:

$$OD_3 = \frac{[\text{nitrite}]_3[\text{NacTRP}]_{in}K_{app}}{1 + K_{app}[\text{nitrite}]_3} \quad \text{ratio } 300/1 \quad (6)$$

Temperature Effect Study. The temperature dependence study was performed at pH 4, and the ratio of [nitrite]/[NacTRP] was 50/1. During this study, temperature was modified in the spectrometer thermostating bath. As a consequence, the optical density at 335 nm changed, showing a shift of the equilibrium. When this had reached a new plateau, temperature was considered stable and measured in the cell. The study was performed in the range of 6.5–42.9 °C. The derivation of the Van't Hoff equation gives the linear equation

$$[d(\ln K_{app})/d(1/T)]_p = -\Delta H_0/R \quad (7)$$

Then the slope of a plot of ln K_{app} vs. $1/T$ at constant

Table II. Comparison, at Some of the pH Values, of Calculated and Measured OD_3 (log K_{app}, ϵ, and OD_3 Determined as Described in the Text)

		pH					
		1	2	2.7	3.1	3.7	4.6
[nitrite]/[NacTRP] = 50/1 and 100/1	log K_{app}	2.6	2.65	2.5	2.36	2.05	1.31
	ϵ	6400	6100	7100	7100	6900	6800
OD_3 ratio 300/1	calcd	0.72	0.69	0.78	0.76	0.66	0.97
	measd	0.72	0.67	0.76	0.74	0.66	0.92
[nitrite]/[NacTRP] = 100/1 and 300/1	log K_{app}	2.57	2.72	2.56	2.43	2.06	1.41
	ϵ	6500	5900	6800	6800	6800	5900
OD_3 ratio 50/1	calcd	0.54	0.54	0.56	0.50	0.33	0.33
	measd	0.54	0.53	0.55	0.49	0.34	0.32
[nitrite]/[NacTRP] = 50/1 and 300/1	log K_{app}	2.58	2.68	2.53	2.40	2.06	1.36
	ϵ	6500	5900	6900	6800	6900	6000
OD_3 ratio 100/1	calcd	0.64	0.60	0.66	0.61	0.48	0.52
	measd	0.63	0.61	0.67	0.62	0.48	0.54

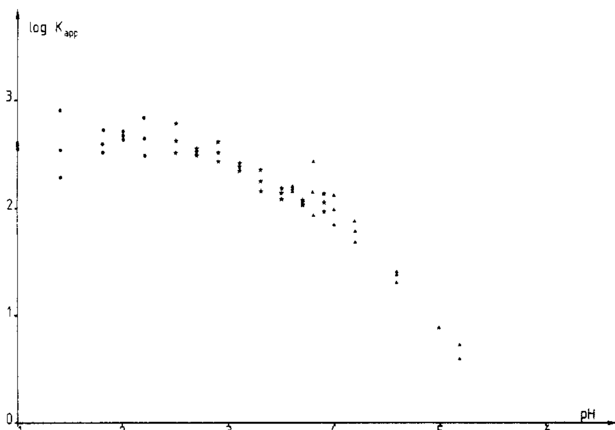

Figure 1. Shift of the equilibrium as a function of the pH: ●, HCl–KCl buffer; ★, aconitic acid–NaOH buffer; ▲, acetic acid–sodium acetate buffer.

pressure (called a Van't Hoff plot) will be equal to $-\Delta H_0/R$. Thus, if the plot is linear, the slope can be utilized to determine the standard molar enthalpy. A value of the standard molar entropy can be obtained by

$$\Delta S_0 = \frac{RT \ln K_{app} + \Delta H_0}{T} \quad (8)$$

RESULTS AND DISCUSSION

Comparison between theoretical and experimental values of OD_3 in the range pH 1–5.2 brings some support to the assumption of an equilibrium. Table II shows some examples of compared values.

The average molar extinction coefficient of NacNOTRP at 335 nm calculated from all the values was found to be 6450 ± 630 cm^{-1} M^{-1}. In order to explain the shift of the equilibrium as a function of the pH (Figure 1), it is necessary to consider the reacting species of nitrite. Under the present experimental conditions the possible species were HNO_2, NO_2^-, N_2O_3, and $H_2NO_2^+$. Kinetic studies made by Kurosky and Hofmann (1972) revealed that nitrosation of the NacTRP was a first-order reaction with respect to $[HNO_2]$ and $[H^+]$. This excludes N_2O_3 and $H_2NO_2^+$ as reacting species as their formation requires two molecules of nitrous acid and two protons, respectively. If nitrous acid is considered as the reacting species, the equilibrium becomes

$$\text{NacTRP} + HNO_2 \overset{K}{\rightleftarrows} \text{NacNOTRP} + H_2O \quad (9)$$

To obtain the apparent constant K_{app}, $[HNO_2]$ can be expressed as a function of [nitrite]

$$[\text{nitrite}] = [HNO_2] + [NO_2^-] + [N_2O_3] + [H_2NO_2^+] \quad (10)$$

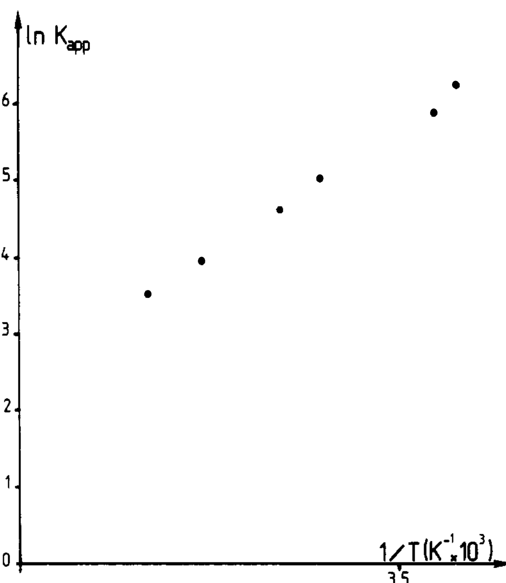

Figure 2. Van't Hoff plot for calculation of ΔH_0 and ΔS_0.

Bunton and Stedman (1959) showed that in these conditions $[N_2O_3] + [H_2NO_2^+] << [HNO_2] + [NO_2^-]$. Therefore

$$[HNO_2] = \frac{[\text{nitrite}]}{1 + K_A/[H^+]} \quad (11)$$

where K_A is the dissociation constant of the nitrous acid equal to 5×10^{-1} M. Then

$$K = \frac{[\text{NacNOTRP}]}{[\text{NacTRP}][\text{nitrite}]/(1 + K_A/[H^+])} \quad (12)$$

Finally

$$K_{app} = \frac{K}{1 + K_A/[H^+]} \quad (13)$$

In order to superimpose the theoretical curve on the experimental one, a nonlinear regression program was followed for the constant K and it was found that by considering the value of K as 455.6 M^{-1} the correlation coefficient was equal to 0.94 with a standard deviation of 0.13. Thus, it can be assumed that the nitrous acid was the reacting species.

The Van't Hoff plot showed a good linearity in the range of 6.5–42.9 °C (Figure 2). Thus, by linear regression it was found $\Delta H_0 = -54\,000$ J. This indicates that the formation of NacNOTRP is an exothermic process. In the same range of temperature $\Delta S_0 = -140$ J·mol^{-1}·K^{-1}.

CONCLUSION

In the present work, evidence has been presented for an equilibrium between nitrous acid, N^{α}-acetyltryptophan, and its nitrosated form. The equilibrium constant is equal to 255.6 M^{-1}; the formation of the NacNOTRP is an exothermic process with $\Delta H_0 = -54000$ J·mol^{-1} in the range of 6.5–42.9 °C. A standard molar entropy can be given equal to −140 J·mol^{-1}·K^{-1}. Thus, tryptophan reactivity is well defined over a large range of pH and temperature.

This helps to determine the cause of the release of nitrite by cured meat and nitrosated myofibrillar proteins, after washing with water (Noël et al., 1981). This can be explained by a shift of the equilibrium caused by the removal of free nitrous acid from the solution. In this case, nitrosotryptophan acts as a reserve of nitrite that is progressively released when the free nitrite concentration decreases in the medium. As it can be seen in the Figure 1 the equilibrium is shifted toward the nitrosotryptophan at low pH. This is in agreement with the results of Noël et al. who showed that labile bound nitrite was in larger amount at a lower pH. Furthermore, this equilibrium explains the "transnitrosation" reaction from nonheme protein tryptophyl residues toward the myoglobin as described by Ito et al. (1983).

Heme groups, which are very reactive toward nitrite, bind to free nitrite. This causes the shift of the equilibrium until the nitrosotryptophan is exhausted. This new transfer mechanism of nitrite from a site to another site of the protein or of another molecule is to be added to the free-radical mechanism suggested by Buglass et al. (1974) in the case of the aromatic amines nitrosation. The mechanism provides the chemical basis for a better classification of the different states of nitrite in cured meat. Usually, nitrite is classified into free and bound fractions. Bound nitrite should be now divided in two forms: irreversibly and reversibly bound. Reversibly bound nitrite can be nitrosotryptophan, but other unknown species cannot be excluded. Usually nitrite extraction from cured meat products is carried out under basic conditions to avoid nitrite destruction. Equilibrium constant variation as a function of the pH provides an additional justification to this method: at basic pH the equilibrium is shifted toward nitrite releasing. Then, the AOAC method of nitrite assay takes in account nitrite reversibly bound on the tryptophan.

ABBREVIATIONS USED

NacTRP = *N*-acetyltryptophan
NacNOTRP = *N*-acetyl-N^1-nitrosotryptophan
OD = optical density
ϵ = molar extinction coefficient for NacNOTRP
K_{app} = apparent equilibrium constant

Registry No. L-NacTRP, 1218-34-4; NacNOTRP, 58332-35-7; NO_2^-, 14797-65-0.

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Persistence and Distribution of Cypermethrin, Deltamethrin, and Fenvalerate in Laying Chickens

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Persistence and distribution of cypermethrin, deltamethrin, and fenvalerate in laying chickens were studied following oral administration of a single-dose mixture containing 10 mg/kg of body weight of each pyrethroid. Residues of the parent compounds were analyzed in fat, skin, blood, heart, brains, liver, kidney, ovary, egg yolk, and egg white 1, 2, 3, 5, 7, and 14 days after oral administration. Residues of the parent compounds in the brains were much higher and more persistent than other tissues investigated. Fenvalerate was the most persistent in all tissues followed by cypermethrin and deltamethrin.

The potential tranfer of pesticides from agricultural uses and from pesticide residues in processed agricultural products used as feed to livestocks and poultry is greatly emphasized. Cypermethrin [α-cyano(3-phenoxybenzyl) 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate], deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl *cis*-(1*R*,3*R*)-2,2-dimethyl-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate], and fenvalerate [cyano-(3-phenoxybenzyl) 4-chloro-α-(1-methylethyl)benzeneacetate] (Figure 1) are among the synthetic pyrethroid

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